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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
08/793,653	02/27/1997	FREDERIC DE SAUVAGE	GTEC113469	5602

7590 06/06/2005

KNOBBE, MARTENS, OLSON & BEAR
620 NEWPORT CENTER DRIVE,
SIXTEENTH FLOOR
NEWPORT BEACH, CA 92660

EXAMINER	
HOWARD, ZACHARY C	
ART UNIT	PAPER NUMBER
1646	

DATE MAILED: 06/06/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	08/793,653	DE SAUVAGE ET AL.	
	Examiner	Art Unit	
	Zachary C. Howard	1646	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *14 March 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *14,16-26 and 28-30* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *14,16-26 and 28-30* is/are rejected.

7)☒ Claim(s) *26* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *27 February 1997* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *1/12/05*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Status of Application, Amendments and/or Claims

The amendment of 3/14/05 has been entered in full. Claims 14, 16, 24, and 25 are amended. Claims 13 and 15 are canceled. New claims 29 and 30 are added.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims 14, 16-26 and 28-30 are under consideration in the instant application.

Submission of Missing References

The examiner thanks the Applicant for the submission of the references that were previously provided with an Information Disclosure Statement but that were not available at time of the previous Office Action. These references have been fully considered.

Withdrawn Objections and/or Rejections

The following page numbers refer to the previous Office Action (9/13/04).

The rejection of claims 24-26 under 35 U.S.C. § 112, first paragraph at pg 2-4 for failing to provide enablement for methods of treatment of type I diabetes or bulimia is withdrawn. Please see the new rejection of claims 24-26 under 35 U.S.C. § 112, first paragraph below. Applicant's argument regarding the previous rejection as they pertain to the new rejection are addressed after the new rejection.

The rejections of claims 13 and 15 under 35 U.S.C. § 102(e) and 103 at pg 5-9 are withdrawn in view of the cancelled claims (3 March 2005). Please see the new claim rejections under 35 U.S.C. § 102(e) and 103 below.

Please see new claim objections and rejections, below.

Claim Objections

Claim 26 depends from claim 13, which is currently cancelled. Appropriate correction is required. The examiner notes that the limitations of claim 13 have been incorporated into claim 14. Therefore, for purposes of prosecution, claim 26 will be interpreted as if it depended from claim 14.

Claim Rejections - 35 USC § 112, 1st paragraph, scope of enablement

Claims 24-26 and 29-30 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for 1) a method of treating a condition associated with a homozygous mutation in the OB (leptin) gene, or a method for eliciting a biological response in rodents, or humans with said homozygous mutation, wherein the biological response is a decrease in food intake or an increase in energy use comprising administering the claimed chimeric polypeptide, and 2) compositions for the treatment of obesity associated with a homozygous mutation in the OB gene, does not reasonably provide enablement for 1) a method of treating a condition associated with the abnormal expression or function of the OB gene or for eliciting a biological response mediated by an OB receptor, or 2) a composition for the treatment of obesity. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

The factors considered when determining if the disclosure satisfies the enablement requirement and whether any necessary experimentation is "undue" include, but are not limited to: 1) nature of the invention, 2) state of the prior art, 3) relative skill of those in the art, 4) level of predictability in the art, 5) existence of working examples, 6) breadth of claims, 7) amount of direction or guidance by the inventor, and 8) quantity of experimentation needed to make or use the invention. In re Wands, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

The specification teaches that an OB-immunoglobulin (OB-Ig) fusion is more effective than native OB at reducing body weight and food intake in obese *ob/ob* female. The term *ob/ob* indicates the mice are homozygous for a mutation in OB gene.

Claim 24 encompasses a method comprising administering to a patient a therapeutically effective amount of a chimeric OB-Ig fusion protein. The method is intended for treating any condition associated with the abnormal expression or function of the OB gene, or for eliciting any biological response mediated by an OB receptor. The specification contemplates that obesity, bulimia, type I diabetes and type II diabetes belong to the genus of conditions associated with abnormal expression of or function of the OB gene. Claim 25 depends from claim 24 and limits said conditions to obesity, bulimia, and type I or type II diabetes. The nature of the invention of Claim 26 is a composition for the treatment of obesity comprising an effective amount of a chimeric polypeptide of claim 14 [see Claim Objections above] in association with a pharmaceutically acceptable carrier. Claims 29 and 30 depend from claim 24 and limit the biological response mediated by an OB receptor to a decrease in food intake or an increase in energy use.

The relevant art teaches that, other than individuals with homozygous mutations in the OB gene, it is not possible to identify obese individuals for which OB treatment is effective in treating obesity. Gale teaches that "rare genetic mutations resulting in leptin or leptin receptor deficiencies in humans also support the notion that leptin plays an important role in satiety...administration of exogenous leptin to these [leptin-deficient] children results in a remarkable decrease in their energy intake and a dramatic loss of fat mass while maintaining lean body mass. Although these studies demonstrate that leptin can be a most effective pharmaceutical preparation for treating obesity in leptin-deficient states, the administration of exogenous leptin fails to reduce adiposity significantly in most cases of human obesity that are characterized by increase adipocyte leptin content and high circulating leptin levels, reflecting a state of leptin resistance" (Gale et al, 2004, Recent Advance in Nutritional Sciences. J Nutr. 2004 Feb;134(2):295-8). Bell-Anderson teaches that in the first human trials where leptin was administered, "there was considerable variability in the amount of weight lost. There were also large variations in reported reductions in energy intake, although those patients who received the largest dose of leptin reported lower energy intake. It appears that in some hyperleptinemic patients, leptin may be useful as a treatment option. The

problem is identifying which patients would benefit from this form of therapy" (Bell-Anderson et al, 2004. Treat Endocrinol. 3(1):11-18).

Therefore with respect to obesity and type II diabetes, while the relevant art supports that obesity (and type II diabetes as it is generally linked with obesity) is characterized by abnormal expression of leptin (encoded by the leptin gene), the relevant art teaches that administration of leptin only predictably treats those patient with abnormally low expression of leptin due to a homozygous mutation in the leptin gene.

With respect to bulimia and type I diabetes, the relevant art does not support that these diseases are associated with abnormal expression of the OB gene. The prior art teaches that type I diabetes "is not associated with obesity" (Ganong, 1989. Review of Medical Physiology, pages 299-300; cited in the previous Office Action). Furthermore, the relevant art does not teach that type I diabetes is associated with low levels of leptin or that administration of leptin would treat type I diabetes. Similarly, the relevant art teaches in a bulimic patient, "although binging and purging episodes were quite frequent, leptin levels remained stable and were neither related to food intake nor to binge episodes" (Abstract of Herpetz et al, May 1998 May; 23(4): 459-653; cited in the previous Office Action), and "although bulimic patients have very bad nutritional behavior, their leptin levels do not appear altered" (Abstract of Calandra, et al, June 2003; 8(2): 130-7; cited in the previous Office Action). To date, the art has not established a connection abnormal expression of the OB gene and type I diabetes, or bulimia.

Therefore, while the specification asserts that the OB-Ig protein of the invention can be used to treat any disorder or condition associated with abnormal OB gene expression, the relevant art teaches that only individuals with a homozygous mutation in the OB gene can be treated with leptin. Furthermore, the relevant art does not teach that the levels of leptin in patients with bulimia or type I diabetes are abnormally low, so that one of skill in the art would predict that said conditions could be treated with leptin.

The specification does not provide any guidance as to what other conditions are associated with the abnormal expression or function of the OB gene, or what biological conditions are associated with an OB receptor other than food intake and energy use.

The quantity of experimentation needed to make and use the invention as claimed would be undue because in order to use the full scope of the claimed invention, a person of skill in the art would need to engage in further experimentation to:

1) identify those obese or type I patients (other than those with homozygous mutations) that can be treated with the OB-Ig of the invention.

2) identify patients with type I diabetes or bulimia that have abnormal expression or function of the OB gene, and then test whether administration of an OB-Ig fusion protein would or would not treat the condition of type I diabetes, or bulimia.

3) identify individuals in which a biological response of a decrease in food intake or an increase in energy use occurs when administered the OB-Ig fusion.

It is acknowledged that the level of skill of those in the art is high, but it is not disclosed and not predictable from the limited teachings of the prior art and specification how the OB-Ig of the present invention could be used to treat a patient with a condition associated with the abnormal expression or function of the OB gene or to elicit a biological response associated with the OB receptor, or how a composition comprising OB-Ig and a pharmaceutically acceptable carrier could be used to treat obesity. There are no methods or working examples disclosing treatment of conditions associated with abnormal expression or function of the OB gene, or disclosing elicitation of a biological response mediate by an OB receptor, with the claimed OB-Ig. Thus the specification fails to teach the skilled artisan how to use the method or composition for treatment or elicitation without resorting to undue experimentation. The specification has not provided the person of ordinary skill in the art the guidance necessary to be able to use the method or composition for the above stated purpose.

Due to the large quantity of experimentation necessary to determine if the method or composition could be used for treatment of conditions associated with abnormal expression or function of the OB gene or for elicitation of a biological response mediate by an OB receptor, the lack of direction/guidance presented in the

specification regarding same, lack of working examples and the teachings of the relevant art regarding the unpredictability in identifying obese patients that respond to OB treatment and the complex nature of the invention, undue experimentation would be required of the skilled artisan to use the claimed invention. What Applicant has provided is a mere wish or plan and an invitation to experiment.

In the response dated 3/14/05 Applicant submits that the method of claims 24 and 25 are enabled for treatment of type I diabetes or bulimia because the specification establishes that there is a nexus between the disorders and the OB protein, and the quantity of experimentation to use the invention would not be undue because this nexus has been established. Applicant further submits that other factors support that the claims are enabled including, the high skill in the art of the practitioners in molecular biology arts at the time the invention was made, the claims are directed to a finite number of identifiable disorders and are therefore not excessively broad, and the methods use a compound that was and is fully enabled, and therefore the method using should also be fully enabled.

Applicant's arguments have been fully considered but are not found persuasive for the following reasons. While the specification states that there is a connection between type I diabetes or bulimia and the OB protein, this asserted connection is not sufficient to enable one of skill in the art to practice the claimed method with these conditions. The examiner agrees the skill in the art was high at the time the invention was made, but it would still require undue experimentation, for the reasons outlined in the new enablement rejection detailed above, to practice the claimed method to treat these conditions. While the claims are limited to a finite number of identifiable disorders, the specification does not enable one of skill in the art to treat even those identifiable disorders. The fact that a product is enabled does not enable each and every possible method of use of the product is also enabled, and the instant method of use is not enabled for the reasons detailed above.

Claim Rejections - 35 USC § 112, 2nd paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 24 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 24 recites the limitation "the chimeric polypeptide" in lines 3-4 . There is insufficient antecedent basis for this limitation in the claim. In this regard, claim 24 would be rendered definite if this limitation was amended to read "a chimeric polypeptide".

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

Claims 24, 25, 29 and 30 are rejected under 35 U.S.C. 102(e) as being clearly anticipated by Pellymounter et al, U.S. Patent Application Publication No. 2003/0203837, filed 5/30/2003 and meriting priority to 11/22/1995 (cited in the previous Office Action).

Claims 24 and 25 each encompass a method comprising administering to a patient a therapeutically effective amount of a chimeric protein comprising a native OB protein with an initiating N-terminal methionine fused to an immunoglobulin heavy chain constant domain sequence. The recitation of "treating a condition associated with the abnormal expression or function of the OB gene or for eliciting a biological response mediated by the OB receptor" in the preamble of the claim is interpreted as an intended

use and bears no accorded patentable weight, except in so far as it limits the "patient" to those with "a condition associated with the abnormal expression or function of the OB gene". Dependent claim 25 limits these conditions to obesity, bulimia, and type I or II diabetes. The specification does not define or limit a "therapeutically effective amount" and therefore the term encompasses any amount that is effective for any therapy.

Pellymounter teaches (page 8, Example 5) administration of a OB protein derivative to a diabetic patient. Pellymounter teaches (paragraph 31) that derivatives of the OB protein include fusion proteins that "may be prepared by attaching polyaminoacids to the OB protein (or analog) moiety. For example, the polyamino acid may be a carrier protein which serves to increase the circulation half-life of the protein. Such polyamino acid may be selected from the group consisting of ...an antibody or portion thereof (such as an antibody constant region, sometimes called "Fc").... As indicated below, the location of attachment of the polyamino acid may be at the N-terminus of the OB protein moiety, or other place, and also may be connected by a chemical "linker" moiety to the OB protein." The term Fc refers to a part of the antibody consisting of only heavy chain constant domain sequences. Pellymounter teaches (paragraph 16, lines 3-4) that the OB protein used may be the human OB protein according to Zhang et al (Reference 37 of the IDS filed 12-3-1998). The sequence taught by Zhang in Figure 6b (page 430) is the "sequence of human OB protein" and includes an initiating N-terminal methionine and a native signal sequence. Pellymounter, in claim 1, teaches "A fusion protein optionally having an N-terminal methionine comprising an antibody constant region or portion thereof attached to the N-terminus of an OB protein." Pellymounter (paragraph 66, lines 1-3) further teaches "One skilled in the art will be able to ascertain effective dosages by administration and observing the desired therapeutic effect." Therefore, Pellymounter teaches a method comprising administering to an diabetic patient a therapeutically effective amount of a chimeric polypeptide that clearly anticipates instant claims 24 and 25.

Claims 29 and 30 encompass a method of increasing energy use or decreasing food intake by administering to a patient a polypeptide of claim 24. The limitations recited in claims 29 and 30 modify the preamble of claim 24 and are therefore

interpreted as an intended use and bear no accorded patentable weight, except in so far as they limit the "patient" to which the chimeric polypeptide will be administered. However, neither the claim nor the specification defines a patient population for which food intake or energy use should be administered; therefore the claim encompasses any type of patient (e.g., obese, diabetic, or healthy). The claim encompasses a method comprising administering to an obese patient a chimeric polypeptide with the limitations of claim 24, and as described above Pellymounter teaches a method with these limitations, and therefore clearly anticipates claims 29 and 30.

Claim Rejections - 35 USC § 103

Claims 14, 16-23, 26, and 28 are rejected under 35 U.S.C. 103 as being unpatentable over Pellymounter et al, U.S. Patent Application Publication No. 2003/0203837, filed 5/30/2003 and meriting priority to 11/22/1995, in view of Capon et al, U.S. Patent No. 5,455,165, published 10/3/1995.

As described above, Pellymounter teaches a chimeric polypeptide comprising the amino acid sequence of a native OB protein, with the N-terminal methionine and with the native signal sequence, fused to Fc region of an antibody, which is an immunoglobulin heavy chain constant domain sequence that comprises the hinge, CH2, and CH3 regions. Pellymounter further teaches (page 2, paragraph 16) a nucleic acid sequence encoding a native human OB protein.

Pellymounter does not teach, that the immunoglobulin constant domain sequence comprises the hinge, CH2, and CH3 regions of an IgG (as in claim 14); or that two chimeric OB polypeptide IgG heavy chain fusion as are linked to each other by at least one disulfide bond (as in claim 16); or the chimeric polypeptide of claim 16 wherein at least one of the heavy chain fusions is associated with an immunoglobulin light chain (as in claim 17); or an isolated nucleic acid molecule encoding the chimeric polypeptide comprising a chimeric polypeptide comprising the amino acid sequence of a native OB protein, with the N-terminal methionine and with the native signal sequence, fused to an immunoglobulin heavy chain constain domain sequence (as in claim 18); or a replicable expression vector comprising said nucleic acid (as in claim 19); or a host

cell transformed with said vector (as in claim 20); or a process of culturing said host cells so that said nucleic acid is expressed and said chimeric polypeptide is produced, and recovering said polypeptide (as in claim 21); said process wherein said host cells are cotransformed with nucleic acid encoding at least two OB protein-immunoglobulin heavy chain constant domain fusions (as in claim 22); or said process wherein said cells are further transformed with nucleic acid encoding at least one immunoglobulin light chain (as in claim 23); or a composition comprising an effective amount of a chimeric polypeptide of claim 14 with a carrier (as in claim 26); or a nucleic acid encoding a chimeric polypeptide comprising a mature native human OB polypeptide fused at its C-terminus, to the N-terminus of an IgG constant domain sequence comprising the hinge, CH2 and CH3 regions (as in claim 28).

Capon teaches general techniques for "compositions and methods for improving the circulating half-life of ligand binding molecules...hybrid immunoglobulin molecules, to methods for making and using these immunoglobulins, and to nucleic acids encoding them." Capon further teaches (col 9, lines 56-58) that "typically, such fusions retain at least functionally active hinge, CH2, and CH3 domains of the constant region of an immunoglobulin heavy chain." Capon further teaches (col 13, 53-55) that "immunoglobulin combining sites and fusion partners are obtained from ... preferably IgG-1." Capon teaches general techniques for "compositions and methods for improving the circulating half-life of ligand binding molecules...hybrid immunoglobulin molecules, to methods for making and using these immunoglobulins, and to nucleic acids encoding them." Capon further teaches (col 9, lines 56-58) that "typically, such fusions retain at least functionally active hinge, CH2, and CH3 domains of the constant region of an immunoglobulin heavy chain." Capon further teaches (col 10, lines 35-65 and col 11, lines 2-3) homodimers consisting of protein-constant domain fusions that are "disulfide bonded in the same fashion as native immunoglobulins". Capon further teaches (same section) that the homodimers can be associated with immunoglobulin light chains; Capon further teaches (col 14, lines 26-38) nucleic acids encoding a protein fused to an immunoglobulin heavy chain constant domain region; Capon further teaches (col 25, lines 7-9) expression hosts transformed with DNA encoding the hybrid which has been

ligated into an expression vector. Capon further teaches (col 28, lines 1-2) that "host cells are transformed with expression vectors of this invention and cultured in conventional nutrient media..." and (col 28, lines 1-2) "the novel polypeptide is recovered and purified from recombinant cell cultures..." Capon further teaches (col 15, line 6-9) "multiply cotransformed cells are used with the above-described recombinant methods to produce polypeptides having multiple specificities..." Capon further teaches (col 14, 61-63) "If multimers are desired then the host cell is transformed with DNA encoding each chain that will make up the multimer"; Capon further teaches (col 5, lines 66-67) formulations of the hybrid immunoglobulins of the invention with pharmacologically acceptable vehicles; and Capon further teaches (col 9, lines 48-49) that "Ordinarily, the ligand binding partner is fused C-terminally to the N-terminus of the constant region of the immunoglobulins..."

It would have been obvious to the person of ordinary skill in the art at the time the invention was made to modify the OB-Fc fusion taught by Pellymounter to use any of the above teachings of Capon. The person of ordinary skill in the art would be motivated to do so because Pellymounter teaches Fc fusions and Capon teaches generic modifications of a hybrid immunoglobulin that can be used to prolong the in vivo plasma half-life of a protein to which the immunoglobulin is fused, and each of the above modifications is taught by Capon as examples of ones that will prolong the half-life of the protein. One of skill in the art would expect success because Pellymounter teaches OB-Fc fusions and Capon teaches all of the techniques necessary to make the modified OB-Fc fusions described above.

Claims 14,16-26, 28, 29 and 30 are rejected under 35 U.S.C. 103(a) as being unpatentable over any one of Zhang et al, Basinski et al ('744 or '886), DiMarchi et al ('954 or '336), in view of Shin et al, or Ashkenazi et al. The basis for this rejection is set forth for claims 14, 16-26 and 28 at pg 7-8 of the previous Office Action of 5/27/1998, and for claim 28 at pg 9 of the previous Office Action 9/13/2004.

Applicant's arguments (3/14/05), as they pertain to the rejections have been fully considered but are not deemed to be persuasive for the following reasons.

Applicants submit that this rejection (set forth 5/27/1998) was withdrawn (3/12/1999) by a previous Examiner in response to the amendments and arguments by Applicants. Applicants note that the USPTO has a duty to apply consistent standards in examining patent applications. Applicants suggest that, as there was no statement that the decision was reversed, the instant Examiner may have overlooked the withdrawal of the rejection made by the previous Examiner. Applicants submit that this position is supported by the statement in the previous Office Action that that the rejection is "is maintained" when in fact the rejection was previously withdrawn. Applicants note MPEP § 706.04 and submit that the previous argument were sufficient to overcome the rejections.

Applicant's arguments have been fully considered but are not found persuasive. The Examiner fully considered the 3/12/1999 withdrawal of the rejection of 5/27/1998 by the previous Examiner. The Examiner of this application has changed and although the USPTO has a duty to apply consistent standards the current Examiner is not bound to repeat mistakes made by the previous Examiner. The Examiner does concede that the statement "is maintained" was not correct in that the rejection had been withdrawn by the previous Examiner. Therefore, the Examiner indicates now for the record the withdrawal of 3/12/1991 is reversed, and the rejection of 5/27/1998 is re-entered.

Applicants also note that the references cited by the Examiner are not as relevant as the references cited by the Applicants in their previous Response. Applicants assert that the references in the previous Office Action (e.g., Shin et al and Ashkenazi et al) are not directed to the relevant protein (OB protein), while the references (Maffei et al, Coleman and Campfield et al) are directed to the relevant protein (OB protein). Applicants submit that the entirety of the prior art must be considered and that one of skill in the art would not have ignored references directly relevant to the protein and questions at hand, and instead look to teachings in unrelated proteins or systems. Applicants disagree with the Examiner's position that the fact that "earlier OB protein studies may not have fully recognized the nature of the interaction

with its cognate receptor, and where such receptors are located, does not detract from the obviousness" (page 7). Applicants argue that to the contrary what one of skill in the art at the time of invention would have believed or not believed concerning OB protein studies and the location of the receptor directly impacts what one of skill in the art would have been motivated to do and would have expected as an outcome. Applicants submit that it was a fact that those of skill in the art believed that these proteins were expressed in the brain and that this fact would suggest that anything that targets the protein would need to be able to pass the blood brain barrier.

Applicant's arguments have been fully considered but are not found persuasive. The examiner agrees that the references Shin et al and Ashkenazi et al are not directed to the relevant reference. However, they were included in the rejection as secondary references used in an obviousness type rejection, and they provide teachings towards making immunoglobulin-protein fusions that are generally applicable to any protein. The primary references used, Zhang et al, Basinski et al ('744 or '886), DiMarchi et al ('954 or '336), discuss the relevant OB protein. The examiner agrees that one of skill in the art would not have ignored the relevant teachings of Maffei et al, Coleman and Campfield et al. However, the teachings of Maffei et al, Coleman and Campfield et al. do not detract from the obviousness of Zhang et al, Basinski et al ('744 or '886), DiMarchi et al ('954 or '336), in view of Shin et al, or Ashkenazi et al. The examiner disagrees with the statement that it was a fact "that that those of skill in the art believed that these proteins were expressed in the brain." Maffei (cited by Applicants) teaches (page 6959) "Mechanisms involving the circumventricular organ and/or specific transporters could permit brain access of a molecule the size of that encoded by the OB gene. However, this hypothesis must considered with caution until the means by which the protein might cross the blood-brain barrier have been identified. Moreover, possible effects on other target organs will need evaluation." Therefore, Maffei teaches clearly states that a brain location of the OB receptor was just a hypothesis and indicates there are alternate hypotheses that it acts on other organs. Furthermore, Maffei suggests a hypothetical scenario by which a molecule the size of OB might enter the brain. There is nothing in this proposed hypothesis that teaches away from a larger OB-Ig fusion entering the

brain by the same mechanism. As cited in the previous Office Action at pg 8, it was known in the art at time the invention was made that transfferin-Ig fusions could penetrate the blood-brain barrier more effectively than transferrin alone. Therefore, Maffei clearly suggests 1) there are possibly ways for large molecules such as OB to enter the brain and 2) OB may work at receptors at other locations besides the brain. Therefore, the Examiner maintains that the prior art, while not fully recognizing the nature of the interaction between OB and its receptor, and where the receptor is located, does not detract from the obviousness of constructing a OB leptin fusion.

New claims 29 and 30 encompass a method of increasing energy use or decreasing food intake by administering to a patient a polypeptide with the same limitations as claim 24. The limitations recited in claims 29 and 30 modify the preamble of claim 24 and are therefore interpreted as an intended use and bear no accorded patentable weight, except in so far as they limit the "patient" to which the chimeric polypeptide will be administered. However, neither the claim nor the specification defines a patient population for which food intake or energy use should be administered; therefore claim encompasses any type of patient (e.g., obese, diabetic, or healthy). Therefore, the claim encompasses a method of comprising administering to an obese patient a chimeric polypeptide with the limitations of claim 24, and are unpatentable in view of the above cited references for the same reasons of record as for claim 24.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Zachary C. Howard whose telephone number is 571-272-2877. The examiner can normally be reached on M-F 9:30 AM - 6:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anthony Caputa can be reached on 571-272-0829. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

zch

Bridget E. Bunner
patent examiner

PTO/SB/08 Equivalent

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Multiple sheets used when necessary)

SHEET 1 OF 1

Application No.	08/793,653
Filing Date	February 27, 1997
First Named Inventor	De Sauvage, Frederic
Art Unit	1646
Examiner	Howard, Zachary C.
Attorney Docket No.	GENENT.052CP2

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Examiner Initials	Cite No.	Document Number *Number - Kind Code (if known)* Example: 1,234,567 B1	Publication Date MM-DD-YYYY	Name of Patentee or Applicant	Pages, Columns, Lines Where Relevant Passages or Relevant Figures Appear
ZH	1	6,355,237	03/12/2002	Snodgrass et al.	

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ZH	2	WO 95/14930				

NON PATENT LITERATURE DOCUMENTS

Examiner Initials	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[1]
ZH	3	Allen, TM. Trends Pharmaceutical Science, 515 (7):215-220, 1994	
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Examiner Signature	Zach Howard	Date Considered	5/25/05

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Notice of References Cited	Application/Control No. 08/793,653	Applicant(s)/Patent Under Reexamination DE SAUVAGE ET AL.	
	Examiner Zachary C. Howard ZH 5/27/05	Art Unit 1646	Page 1 of 1

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	U	Gale et al, 2004, Recent Advance in Nutritional Sciences. J Nutr. 2004 Feb;134(2):295-8.
	V	Bell-Anderson et al, 2004. Treat Endocrinol. 3(1):11-18.
	W	
	X	

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Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Energy Homeostasis, Obesity and Eating Disorders: Recent Advances in Endocrinology[1]

Susan M. Gale, V. Daniel Castracane and Christos S. Mantzoros*[2]

*Diagnostic Systems Laboratories, Incorporated, Webster, TX 77598 and *Beth Israel Deaconess Medical Center, Department of Endocrinology Diabetes and Metabolism, RN 325, Boston, MA 02215*

ABSTRACT Health problems resulting from obesity could offset many of the recent health gains achieved by modern medicine, and obesity may replace tobacco as the number one health risk for developed societies. An estimated 300,000 deaths per year and significant morbidity are directly attributable to obesity, mainly due to heart disease, diabetes, cancer, asthma, sleep apnea, arthritis, reproductive complications and psychological disturbances. In parallel with the increasing prevalence of obesity, there has been a dramatic increase in the number of scientific and clinical studies on the control of energy homeostasis and the pathogenesis of obesity to further our understanding of energy balance. It is now recognized that there are many central and peripheral factors involved in energy homeostasis, and it is expected that the understanding of these mechanisms should lead to effective treatments for the control of obesity. This brief review discusses the potential role of several recently discovered molecular pathways involved in the control of energy homeostasis, obesity and eating disorders. J. Nutr. 134: 295–298, 2004.

KEY WORDS: • obesity • energy homeostasis • leptin • ghrelin • insulin • adiponectin • resistin • peptide YY3–36

In the early 1950s, it was first postulated that food intake is closely linked to the amount of stored energy (fat mass) in the body. During the 1970s and 80s, gut peptide cholecystokinin, bombesin, gastrin-releasing peptide, neuromedin B (1) and glucagon (2) were identified as "immediate" satiety signals released from the gastrointestinal tract in response to the presence of food. During the 1990s, leptin was recognized as a longer-term adiposity signal, secreted in proportion to body fat stores. Moreover, in addition to modulating immediate peripheral satiety signals, insulin and leptin were shown to directly target the central nervous system and inhibit food intake (3). The currently accepted model of energy homeostasis proposes that peripheral signals become integrated with other regulators of food intake, such as the presence of food, habits or social behavior. Similarly, meal termination may be governed by extrinsic factors and intrinsic factors, the latter including signals generated in the organism in response to the consumption of food.

Leptin. The discovery of leptin, the product of the obese gene (4), and soon thereafter, the characterization of the leptin receptor (5) and the circulating leptin binding protein (soluble extracellular domain of the leptin receptor) (6) renewed interest in the hormonal regulation of energy balance. Leptin is a 16-kDa glycosylated protein of 146 amino acids produced predominantly by adipose tissue, although low levels of expression were also detected in the hypothalamus, pituitary, placenta, skeletal muscle, and gastric and mammary epithelia (7). Leptin may play a role in many diverse physiologic processes, but it is primarily involved in energy homeostasis and satiety. Leptin levels in the circulation are increased in proportion to fat mass, and circulating leptin conveys information to the hypothalamus regarding the amount of energy stored in adipose tissue, suppressing appetite and affecting energy expenditure (8).

Initial studies investigating the physiologic role of leptin in mice demonstrated that leptin was directly involved in the regulation of satiety, energy balance and feeding behavior. *Ob/ob* mice, which do not produce functional leptin, become enormously obese when feeding regimens allow ad libitum consumption; they reach four times normal body weight compared with controls. The administration of leptin can reverse this weight gain in this and other mouse models of obesity, indicating that leptin plays an important role in the regulation of food intake.

Rare genetic mutations resulting in leptin or leptin receptor deficiencies in humans also support the notion that leptin plays an important role in satiety. Leptin-deficient children exhibit ravenous feeding behavior and develop extreme obesity. Administration of exogenous leptin to these children results in a remarkable decrease in their energy intake and a dramatic loss of fat mass while maintaining lean body mass (9,10). Although these studies demonstrate that leptin can be a most effective pharmaceutical preparation for treating obesity in leptin-deficient states, the administration of exogenous leptin fails to reduce adiposity significantly in most cases of human obesity that are characterized by increased adipocyte leptin content and high circulating leptin levels, reflecting a state of leptin resistance. The mechanisms underlying leptin resistance in obese humans may include defective transport of leptin into the brain, and/or reduced hypothalamic leptin signaling, which is in part due to up-regulation of specific inhibitors of leptin signaling. The pathogenesis of leptin resistance is currently under intense investigation, and it is expected that elucidation of the mechanisms underlying leptin resistance may lead to the development of new therapeutic options for the treatment of obesity.

Weight-loss programs are well known to be ineffective long term, with most individuals regaining any weight lost within a short period of time, and it has been proposed that the corre-

[1] Manuscript received 14 October 2003.
[2] To whom correspondence should be addressed.
E-mail: cmantzor@bidmc.harvard.edu.

sponding decline in serum leptin levels due to the loss in fat mass may contribute to the inability of these subjects to maintain their weight loss. Exogenous leptin administration to replace leptin levels to preweight-loss levels prevented the regaining of weight and promoted loss of fat mass while preserving fat-free mass (11) in a small group of subjects participating in a weight loss program, but these findings have to be replicated by larger studies.

In this context, it was shown recently that decreasing leptin levels in response to food deprivation are responsible for the starvation-induced suppression of the hypothalamic-pituitary-gonadal axis (12), as well as the malfunction of several other neuroendocrine axes. Thus, it seems that leptin may act as the critical link between adipose tissue and not only hypothalamic centers regulating energy homeostasis but also the reproductive system, indicating whether adequate energy reserves are present for normal reproductive function (13).

Insulin. Insulin and leptin share many properties as adiposity signals. Although insulin is secreted from pancreatic β cells rather than adipocytes, the circulating concentration of insulin is also proportional to adiposity (14). In a manner similar to leptin, insulin also crosses the blood-brain barrier and interacts with specific receptors in the arcuate nucleus of the hypothalamus (15). Moreover, through interaction with specific neurons in the arcuate nucleus, both leptin and insulin reduce food intake and body weight in a dose-dependent manner when administered directly into the central nervous system (16). Unlike leptin, insulin secretion from the pancreas is stimulated acutely in response to meals, whereas leptin is not, and it has been shown that prolonged hyperinsulinemia may stimulate the secretion of leptin. Finally, obesity in the vast majority of obese humans is associated with both hyperinsulinemia and hyperleptinemia, which are indicative of insulin and leptin resistance, respectively. Adiponectin and resistin are two recently identified molecules that were reported to enhance or impair insulin sensitivity, respectively.

Adiponectin. Adiponectin, also called gelatin-binding protein-28, apM1, AdipoQ and Acrp30, is a 244-amino acid protein expressed and secreted exclusively from white adipose tissue. Adiponectin circulates at high levels in human plasma as a homopolymer comprising up to 18 monomeric units; the basic unit is a homotrimer, whereas the monomeric unit has never been described under natural conditions.

Adiponectin acts as an insulin-sensitizing hormone whose blood concentrations are reduced in obesity and type 2 diabetes. Administration of recombinant adiponectin to rodents increases glucose uptake and fat oxidation in muscle, reduces fatty acid uptake and hepatic glucose production in liver, and improves whole-body insulin resistance (17). In rhesus monkeys, the decrease in plasma adiponectin levels parallels the development of insulin resistance and type 2 diabetes (18). Moreover, thiazolidinediones, drugs that enhance insulin sensitivity, increase plasma adiponectin and mRNA levels in mice (19). In support of these findings, adiponectin was shown to be negatively correlated with body weight, body fat mass and insulin levels in humans.

Resistin. Resistin, also called "adipose-tissue-specific secretory factor" (20) and "FIZZ3" (21), is a unique signaling polypeptide secreted by adipocytes. The resistin gene encodes a 114-amino acid polypeptide with a 20-amino acid signal sequence, and is secreted as a 94-amino acid polypeptide with 11 cysteine residues. Resistin is secreted as a dimer, with a single di-cysteine residue required for dimerization, whereas the remaining 10 cysteine residues are involved in determining the structure of the resistin monomeric unit. Currently available data from studies in rodents are conflicting, however, and studies in humans are still required to confirm a role for resistin in regulating insulin sensitivity.

In 2001 Steppan and colleagues (22) reported that resistin secretion is decreased by the antidiabetic drug rosaglitazone and is increased in diet-induced and genetic mouse models of obesity. Moreover, administration of anti-resistin antibody improves blood sugar and insulin action in obese mice, and administration of recombinant resistin impairs glucose tolerance and insulin action in normal mice. These observations have not been confirmed by other investigators, however; thus, the role of resistin in mice remains controversial. Studies in humans to fully elucidate the role of resistin are still required, but initial observational and interventional studies have failed to support a role for circulating resistin in regulating insulin resistance in humans (23).

Ghrelin. Ghrelin was originally discovered by Kojima and co-workers (24) during their search for an endogenous growth hormone secretagogue. This hormone is a 28-amino acid lipophilic peptide with a labile octanoic acid side chain at the serine residue three, which is expressed primarily in specialized enterochromaffin cells located mainly in the mucosa of the fundus of the stomach (24). Ghrelin has metabolic effects opposite to those of leptin. It stimulates food intake, enhances the use of carbohydrates and reduces fat utilization, increases gastric motility and acid secretion and reduces locomotor activity.

Although ghrelin has potent growth hormone–releasing properties comparable to those of growth hormone–releasing hormone (24), it also has powerful effects that are independent of growth hormone (25). Administration of ghrelin peripherally or centrally into the cerebral ventricles induces weight gain in rodents (26); in humans, ghrelin levels peak before each meal and then fall to lower levels immediately upon food consumption.

In humans, circulating ghrelin levels are decreased in acute states of positive energy balance and in chronic obesity, but elevated during fasting and in anorexia nervosa. These data further support the hypothesis that the secretion of ghrelin not only has effects opposite to leptin, but is also regulated antipodal to leptin. Whether increased ghrelin levels in anorexia nervosa reflect a pathophysiologic state of ghrelin resistance analogous to that of leptin resistance in obesity remains to be elucidated. In addition, the development of a ghrelin antagonist, or the development of a mechanism to inhibit ghrelin release to control appetite, may be an important pharmaceutical development for the management of obesity.

Finally, recent evidence suggests that ghrelin may play a role in reproductive function, a scenario that is analogous to the elucidation of a role of leptin in the control of reproductive function (13).

Peptide YY3–36. Another gastrointestinal tract–derived peptide, which was first identified in 1980 (27) and has only recently been appreciated as a hormonal regulator of appetite, is peptide YY3–36 (28). Peptide YY3–36 is produced by the gut in response to the presence of food and is found to decrease food intake. In common with leptin, this peptide has been shown to cross the blood-brain barrier and act on the arcuate nucleus of the hypothalamus, stimulating neurons that create a sensation of satiety and inhibiting neurons that stimulate feeding behavior (28). In a recent study (29), the effect of an infusion of peptide YY3–36 on appetite and food intake in 12 obese and 12 lean subjects demonstrated that, unlike leptin,

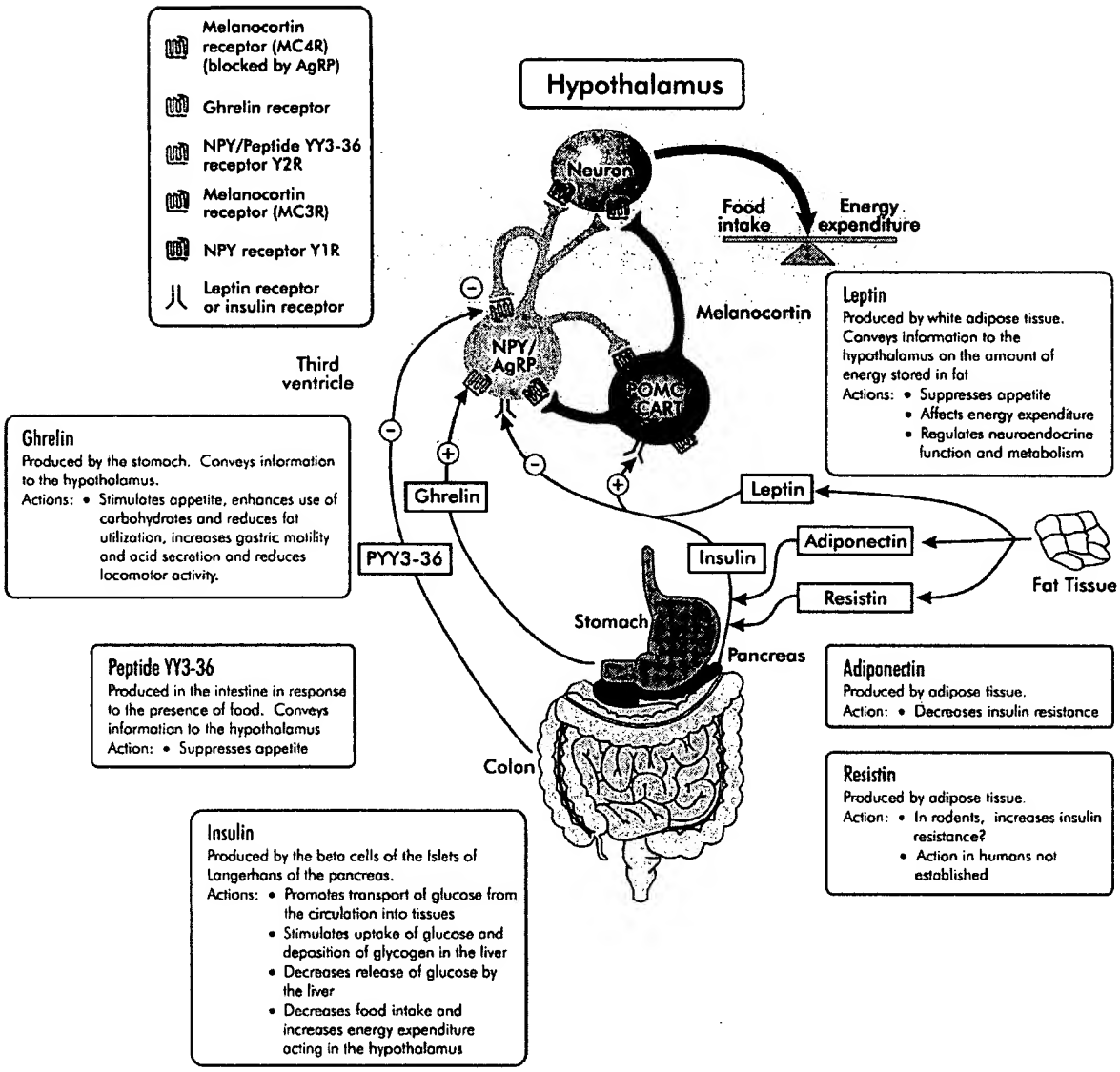

FIGURE 1 The link between the periphery and the brain: endocrine and neuronal interaction in the regulation of energy homeostasis and appetite.

there was no evidence of resistance to peptide YY3–36 in obese subjects. Endogenous levels of peptide YY3–36 were low in the obese subjects, suggesting that peptide YY3–36 deficiency may contribute to the pathogenesis of obesity, and infusion of peptide YY3–36 significantly decreased the cumulative 24-h energy intake in both obese and lean subjects. The same group of investigators recently reported that pancreatic polypeptide has similar effects on energy homeostasis. These results are certainly of great interest and warrant further investigation.

The Link between the Periphery and the Brain: Metabolic Circuitry in the Hypothalamus. It is currently accepted that appetite is regulated by an interplay of hormonal and neural mechanisms, discussed comprehensively in several recent publications (30,31). In brief, the arcuate nucleus of the hypothalamus houses two opposing sets of neuronal circuitry, i.e., an appetite-stimulating circuit and an appetite-inhibiting circuit (see Fig. 1). The two circuits send signals mainly to the paraventricular nucleus (PVN)[3] but also to other nuclei of the hypothalamus, which then directly modulate feeding behavior. The appetite-stimulatory and appetite-inhibitory circuits are influenced by peripheral hormonal signals that are able to cross the blood-brain barrier, such as leptin, insulin, ghrelin and peptide YY3–36.

The appetite-stimulatory circuit produces two neurotransmitters, i.e., neuropeptide Y (NPY) and agouti-related peptide (AgRP), both of which promote appetite. NPY directly signals to the PVN to promote feeding behavior, whereas AgRP acts indirectly by blocking the melanocortin type 4 receptor, an appetite-inhibitory receptor in the PVN. The appetite-inhibitory circuit includes cocaine- and amphetamine-regulated transcript and mainly proopiomelanocortin, which produces α-melanocyte–stimulating hormone (α-MSH). The latter hormone operates mainly through the melanocortin type 4 recep-

[3] Abbreviations used: AgRP, agouti-related peptide; α-MSH, α-melanocyte–stimulating hormone; NPY, neuropeptide Y; PVN, paraventricular nucleus.

tor (and to a lesser extent, through the melanocortin type 3 receptor) to inhibit appetite (31).

Leptin and insulin alike trigger the appetite-inhibitory circuit through up-regulation of α-MSH and inhibit the appetite-stimulatory neuron by suppressing NPY and AgRP mRNA expression in the hypothalamus, whereas ghrelin has largely the opposite effect.

In studies in animals, peptide YY3–36 released from the intestinal tract after the ingestion of food, inhibits the hypothalamic NPY- and AgRP-expressing neurons, thereby disinhibiting adjacent proopiomelanocortin-expressing neurons and decreasing food intake.

Ghrelin and leptin receptors were also demonstrated in brainstem nuclei, and direct injection of leptin into the dorsal vagal complex reduces food intake and body weight in rats (32). Thus, although it has been established that the hypothalamus is central to the control of energy balance, accumulating evidence suggests that neural circuits originating in the caudal brainstem are also involved (33), and this area represents the focus of intense research efforts at this time.

Concluding Remarks. This brief review has covered recent advances in the area of molecules regulating energy homeostasis and obesity. Other related hormones are expected to be added to this list in the near future as well as synthetic analogs developed by the pharmaceutical industry for the management of obesity. Further studies in obesity regulation will require the performance of appropriate animal studies to test the effectiveness of future putative antiobesity drugs as well as well-designed trials in humans to provide a better understanding of the role of any new antiobesity treatments.

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LEADING ARTICLE

Treat Endocrinol 2004; 3 (1): 11-18
1175-6349/04/0001 0011/$31.00/0

Leptin as a Potential Treatment for Obesity

Progress to Date

Kim S. Bell-Anderson and *Janet M. Bryson*

Human Nutrition Unit, School of Molecular and Microbial Biosciences, University of Sydney, Sydney, Australia

Abstract

Despite significant reductions in the consumption of dietary fat, the prevalence of obesity is steadily rising in western civilization. Of particular concern is the recent epidemic of childhood obesity, which is expected to increase the incidence of obesity-related disorders. The *obese* gene (*ob*) protein product leptin is a hormone that is secreted from adipocytes and functions to suppress appetite and increase energy expenditure. Leptin is an attractive candidate for the treatment of obesity as it is an endogenous protein and has been demonstrated to have potent effects on bodyweight and adiposity in rodents.

Whereas leptin has been successfully used in the treatment of leptin-deficient obese patients, trials in hyperleptinemic obese patients have yielded variable results. Long-acting leptins have been tried but with no greater success. Other strategies including the use of leptin analogs and other factors that bypass normal leptin delivery systems are being developed. Identifying the mechanisms at the molecular level by which leptin functions will create new avenues for pharmaceutical targeting to simulate the intracellular effects of leptin.

1. Obesity

1.1 The Problem of Obesity

According to WHO, "obesity is becoming one of the most important contributors to ill health".[1] The alarming increase in the prevalence of obesity, in particular childhood obesity (a doubling over the past 20 years), has created a greater urgency for biomedical research to produce an effective treatment for this disorder. Obesity is a chronic disease characterized by hypertrophy and hyperplasia of fat cells leading to an increased risk of developing type 2 diabetes mellitus, heart disease, and metabolic syndrome X.[2-4] Patients with obesity have a reduced quality of life and an increased risk of psychological disorders.[5] The disease is also a burden on the public health system and society.

1.2 Causes of Obesity

Obesity is diagnosed in humans as a body mass index (BMI) >30 kg/m^2.[6] The underlying cause of obesity is multifactorial and it is likely to be a combination of genetic, environmental, and psychosocial factors that determines the long-term balance between energy intake and energy expenditure. Disruptions in the balance of this system over time can be cumulative and result in positive energy gain and obesity. To a large extent, body adiposity is centrally controlled at the level of the hypothalamus, which is responsible for integrating appetite, metabolic rate, and physical activity. The hypothalamus is linked to adipose depots by the 'adipostat' leptin, a hormone that strongly correlates with body fat mass. Leptin is an appropriate candidate for the treatment of obesity, as it has been shown to have potent effects on the suppression of appetite and stimulation of energy expenditure in rodents and humans.[7]

2. Leptin

2.1 Discovery of Leptin

Discovery of the adipostat hormone dates back to the 1970s with the pioneering work of Coleman,[8] who performed parabiosis studies in mice. Coleman surgically joined blood vessels of genetically obese mice (*ob/ob*) to those of normal mice and observed a complete reversal of the obese mouse phenotype. Furthermore, when *ob/ob* mice or normal mice were paired with genetically diabetic mice (*db/db*), they rapidly became emaciated and died.[9] From these data, Coleman hypothesized the existence of a blood-born 'satiety factor' that was deficient in *ob/ob* mice but overexpressed in *db/db* mice. It was not until 1994 that this factor was

cloned and identified as the *obese* gene (*ob*) product, leptin.[10] The mutation in the *db/db* mice was later identified as abnormal splicing of the gene encoding the leptin receptor.[11]

2.2 Properties of Leptin

Leptin is a 16 kDa serum protein with a half-life of 25–40 minutes. It is synthesized and secreted mainly from adipocytes but has also been found in placenta, heart, ovaries, mammary glands, and gastric endothelium.[12] The majority of data on leptin have been obtained through animal studies, particularly in rodents, which share 83–84% leptin gene sequence homology with humans.

Leptin gene expression is regulated by hormonal and nutritional status. Leptin levels in the circulatory system are increased after a meal; this increase is thought to be due to the direct stimulation of *ob* gene expression and/or leptin secretion from adipose tissue by glucose and insulin. In the fasted state, plasma leptin levels are much lower and are more reflective of body fat mass, so that leptin directly correlates with adiposity in humans. Leptin expression may also be increased by corticosteroids and decreased in response to β-adrenoceptor agonists, cyclic adenosine monophosphate, and thiazolidinediones.[13] Leptin has a myriad of functions mediated predominantly by its endocrine effects, discussed in section 3 (see also figure 1) and detailed in a review by Wauters et al.[14]

2.3 Leptin Receptors

The effects of leptin are mediated by leptin receptors predominantly found in the hypothalamus (choroid plexus, arcuate, and dorsomedial nuclei); however, leptin receptors are also present and expressed in most rodent and human tissues.[15] In rodents, the leptin receptor has six splice variants (Ob-Ra, -Rb, -Rc, -Rd, -Re, and -Rf), of which Ob-Ra and Ob-Rb are the predominant forms.[11] Ob-Ra contains a short intracellular domain and is thought to function as a leptin carrier across the blood-brain barrier.[16] Ob-Rb, the long form of the leptin receptor, contains a long intracellular domain that is able to activate intracellular signaling proteins upon ligand binding (figure 2). Ob-Rb is homologous to class 1 cytokine receptors, which act through the signaling proteins janus kinase (JAK) and signal transducers and activators of transcription (STAT).[17,18] Leptin signal transduction results in STAT3 phosphorylation and activation, after which STAT3 is translocated to the nucleus where it acts as a transcription factor for specific gene activation.[19,20] The leptin receptor is essential for the central and peripheral effects of leptin, as mice

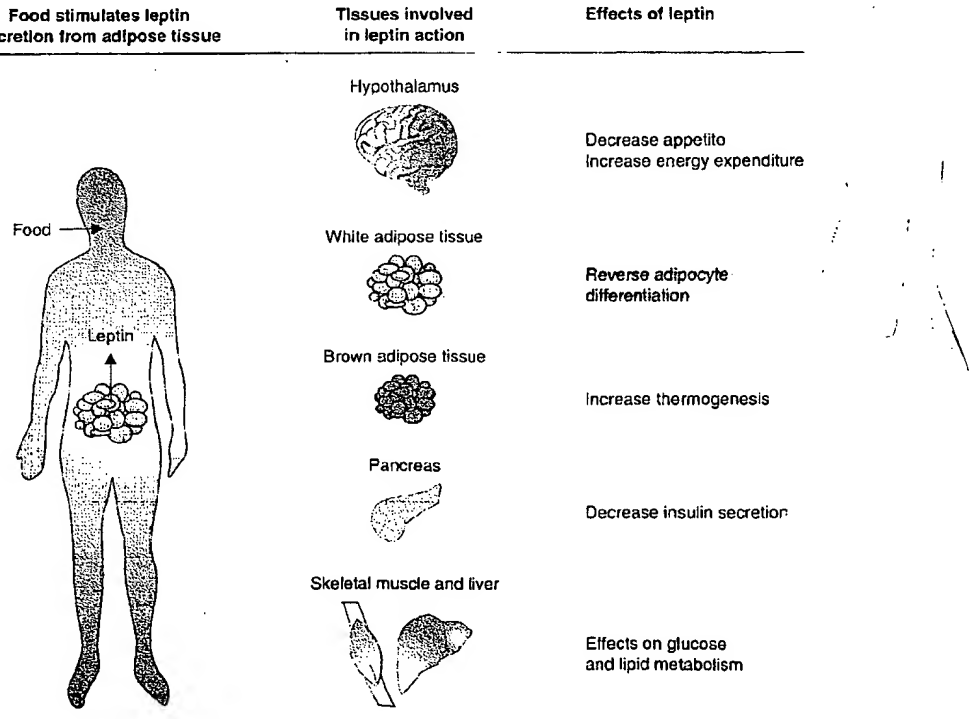

Fig. 1. The effects of leptin are mainly mediated via the hypothalamus, which regulates appetite and energy expenditure. Leptin also has direct effects on some peripheral tissues.

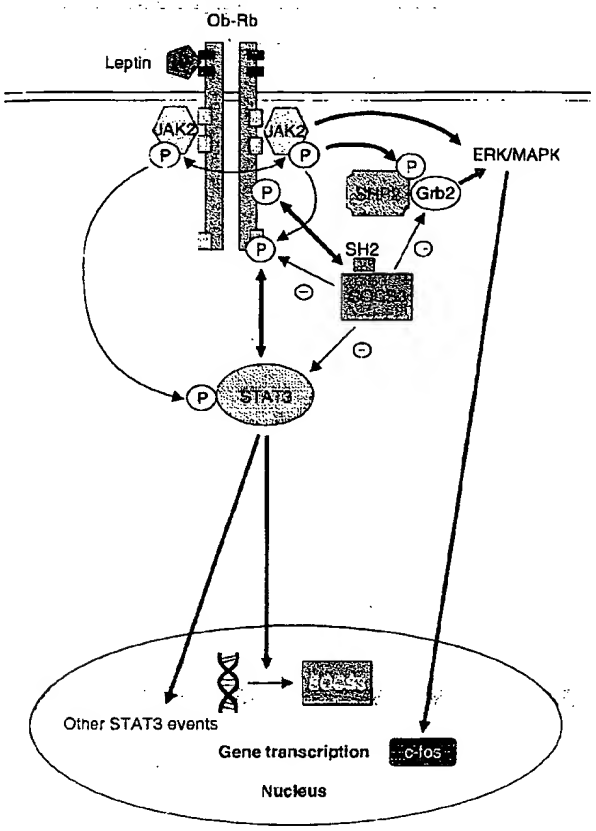

Fig. 2. Leptin signal transduction through the long form of the leptin receptor (Ob-Rb). Leptin binding stimulates tyrosine phosphorylation (P) on janus kinase (JAK)-2 and Ob-Rb, initiating a cascade of events to activate mitogen-activated protein kinase (MAPK) and signal transducers and activators of transcription (STAT)-3 pathways. The main effects of leptin are mediated through these pathways to activate specific gene expression. **ERK** = extracellular regulated kinase; **Grb2** = growth factor receptor binding protein 2; **SH2** = Src homology domain; **SHP2** = SH2 domain containing phosphatase; **SOCS** = suppressor of cytokine signaling.

with a mutation in the gene encoding the leptin receptor (*db/db*) are profoundly obese and do not respond to leptin therapy.[21]

3. Leptin Effects in Rodents

3.1 Animal Models with Mutations in the Leptin or Leptin Receptor Gene

Animal models of leptin deficiency (*ob/ob* mice) and leptin resistance (leptin receptor mutant mice [*db/db*] and rats [*fa/fa*]) have significantly contributed to the elucidation of the actions of leptin. Both models display similar metabolic abnormalities such as increased food intake, obesity, increased fatty liver, insulin resistance, and diabetes. Leptin treatment of *ob/ob* mice significantly decreases bodyweight through the reduction of adiposity, but it has no effect on *db/db* mice with defects in the leptin receptor.[22,23]

From these and similar studies in rodents, it is well established that leptin exerts a centrally mediated dose-dependent reduction in feeding;[24] even a 35 amino acid leptin fragment achieves a similar effect.[25] However, the effects of leptin on bodyweight are not solely attributable to a reduction in appetite, as pair-feeding does not cause weight loss to the same extent.[26,27] Therefore, leptin must also have an effect on energy expenditure. Leptin reduces the respiratory quotient (RQ) in *ob/ob* mice, indicating an increase in fat oxidation and metabolic rate.[22,23,28]

The *ob/ob* mice phenotype also displays perturbations in thyroidal, gonadal, immune, and adrenal functions comparable to those exhibited in humans with congenital leptin deficiency.[29] Furthermore, administration of leptin to *ob/ob* mice is able to reverse all aspects of the phenotype.

3.2 Normal Rats

In normal rats, subcutaneous (SC) injection of leptin for 35 days (250 μg/day) reduced fat content from 28g to 4g without changing lean mass. This was associated with increased fat oxidation but only reduced food intake for the first 10 days.[30] In pair-fed rats, peripheral administration for 8 days (0.5 mg/kg/day) decreased visceral fat adiposity by 62%.[31] Adenovirus-mediated overexpression of leptin in normal rats (increasing plasma leptin levels to >20 μg/L compared with 1 μg/L in controls) results in rapid depletion of fat stores (32g weight loss over 6 days) and the suppression of appetite (30–50% reduction).[32]

Leptin treatment of starved mice indicates that leptin is able to prevent some starvation-induced changes in thyroxine, gonadotropins, and corticosterone.[33] Leptin also reverses the immunosuppressive effects of acute starvation through regulation of the proliferation of T cells.[34]

3.3 Central Pathways Activated by Leptin

Centrally, leptin suppresses appetite and enhances energy expenditure through inhibition of neuropeptide Y (NPY) and agouti-related protein (AgRP) and stimulation of cocaine and amphetamine-regulated transcript (CART), pro-opiomelanocortin (POMC; via α-melanocyte-stimulating hormone [αMSH]), and corticotropin-releasing hormone (CRH) in the hypothalamus.[35,36] Leptin may also act to control food intake and energy expenditure via activation of the sympathetic nervous system, as central leptin administration increases catecholamine secretion.[37]

3.4 Lipodystrophy

In animal models of lipodystrophy where there is partial or total loss of white adipose tissue, circulating leptin levels are severely depressed.[38] These animals are unable to store lipids in the appropriate adipose depots; instead, fatty acids are redirected into storage in peripheral tissues such as liver and skeletal muscle resulting in insulin resistance.[39,40] The lipodystrophic phenotype can be saved by surgical transplantation of adipose tissue or pharmacological doses of leptin.[34,41] However, transplantation of fat lacking leptin (i.e. from *ob/ob* mice) does not reverse the metabolic abnormalities *in vivo*.[42] This research indicates that leptin is essential for normal adipose tissue development, even though its main function is to limit adiposity.

3.5 Peripheral Effects of Leptin

3.5.1 White Adipose Tissue

How does leptin reduce fat mass? Leptin appears to reverse adipocyte differentiation, thereby reducing fat storage in cells and increasing fatty acid oxidation. Leptin treatment in rodents is associated with important alterations in peroxisome proliferator activated receptor (PPAR) gene expression in adipocytes. PPARγ, an important transcription factor involved in adipocyte differentiation and regulation of lipogenesis, is significantly downregulated, whereas PPARα is upregulated along with its downstream proteins, which are involved in fatty acid oxidation.[32] Leptin has been repeatedly shown to decrease the expression of lipogenic enzymes such as fatty acid synthase.[43,44]

3.5.2 Brown Adipose Tissue

It appears that leptin may also increase energy expenditure by increasing thermogenesis in brown adipose tissue (BAT). In normal rats, leptin-induced increases in fat oxidation are associated with increased BAT uncoupling protein (UCP)-1 gene expression.[45] In *ob/ob* mice, leptin increased UCP1 and UCP3 messenger RNA in BAT.[46]

3.5.3 Pancreatic β Cells

Other peripheral effects of leptin have been reported in pancreatic β cells in both rodents and humans. Specifically, leptin directly inhibits glucose-stimulated insulin secretion from β cells,[42,47] perhaps by activation of adenosine triphosphate-sensitive potassium channels and/or reduction of lipotoxicity of β cells.[48,49] A leptin-mediated reduction in the amount of insulin secreted may result in lower levels of whole body lipogenesis and would therefore favor fat oxidation.

4. Leptin Effects in Humans

4.1 In Leptin-Deficient Obesity

There are few reports of the successful use of leptin in the treatment of human obesity. The first report of leptin therapy in a child with congenital leptin deficiency (due to a mutation in the leptin gene) showed that a single daily dose of SC metreleptin (recombinant methionyl human leptin, r-MetHuLeptin; 0.028 mg/kg lean mass/day) resulted in weight loss of 16.4kg in 12 months.[50] Of the total weight lost, 95% was attributable to loss of body fat. There was a marked reduction in food intake, accompanied by a change in eating behavior. Recently, long-term leptin treatment of three morbidly obese children with congenital leptin deficiency was reported.[51] Accompanying significant reductions in appetite, fat mass, and circulating insulin and lipid levels, leptin increased plasma thyroid hormones and facilitated normal pubertal development. Leptin was also able to reverse the reduction in CD4+ T cells and impaired T-cell proliferation and cytokine release, indicating that leptin has many physiological roles including immune and reproductive function.[51]

Leptin treatment has been reported in three leptin-deficient adults who also have mutations in the leptin gene.[52] In these three patients, leptin was given at dosages ranging from 0.019 to 0.042 mg/kg/day for 3 months. Leptin caused an average weight loss of 18kg, of which 85% was fat loss. This reduction in body fat was accompanied by a 58% reduction in energy intake and an increase of 62% in 24-hour fat oxidation. These trials confirmed that exogenous leptin administration in humans is able to induce weight loss, with this weight loss possibly due to the dual effects of leptin therapy (i.e. energy intake and fat oxidation).

Recently, Farooqi et al.[53] have shown that patients who are heterozygous for the mutation in the leptin gene have relatively low leptin levels for their degree of adiposity; these researchers have suggested that such patients may benefit from leptin treatment. Other subgroups of obese patients with lower-than-expected leptin levels may also be suitable candidates for leptin therapy.

4.2 In Hyperleptinemic Obesity

Obesity in humans as a result of defects in the leptin gene is extremely rare, as the majority of obese patients have higher than normal circulating leptin levels. In only a few patients who have hyperleptinemic obesity is the hyperleptinemia due to a defect in the leptin receptor.[54,55] Increases in leptin levels in the cerebrospinal fluid (CSF) of obese patients are disproportionally lower than the increases seen in plasma levels, suggesting that a major contributing factor to hyperleptinemia in most obese patients is leptin resistance caused by defective transport into the central

nervous system.[56,57] Therefore, the use of high-dose recombinant leptin therapy was seen as a possible treatment regimen for the majority of obese patients. However, reports so far suggest that this form of therapy is not always successful in achieving weight loss.

In the first published trials, metreleptin was given daily by SC injection at doses ranging from 0.01 to 0.30 mg/kg.[58] In the group receiving the highest dose, weight loss averaged 7.1kg over 20 weeks, with 95% of this weight loss due to a reduction in fat mass. However, there was considerable variability in the amount of weight lost. There were also large variations in reported reductions in energy intake, although those patients who received the largest dose of leptin reported lower energy intake. It appears that in some hyperleptinemic patients, leptin may be useful as a treatment option. The problem is identifying which patients would benefit from this form of therapy.

Extended treatment with low-dose leptin after weight loss may have a potential role in the maintenance of weight reduction. After significant weight loss in humans, energy expenditure and plasma leptin and thyroid hormone levels are typically reduced;[59] restoring these leptin levels with low-dose leptin is able to reverse these endocrine changes. Administration of leptin (0.08–0.14 mg/kg fat mass, twice daily for 5 weeks) increased 24-hour energy expenditure and circulating liothyronine (triiodothyronine) and thyroxine levels while sustaining the reduction in bodyweight in adults (BMI range after weight loss 19.7–59.4 kg/m^2).[60]

In order to show that the recombinant leptin was reaching target sites in the hypothalamus, CSF leptin levels were measured by Fujioka et al.[61] in a separate cohort of obese patients who were given metreleptin at a dosage of 1 mg/kg/day for 1 week by continuous SC infusion. Both serum and CSF leptin levels were higher in the leptin-treated than in the placebo-treated patients, suggesting that exogenously administered leptin was able to cross the blood-brain barrier. However, again there was considerable variability in the CSF leptin levels, suggesting that variability in the response of obese patients to leptin treatment may be the result of variations in the ability of leptin to reach its site(s) of action.

4.3 Trials with Long-Acting Leptins

In the larger trial mentioned in section 4.2, serum leptin levels peaked about 4 hours after injection, which was given before 11.00 each morning. The half-life of leptin in the serum was relatively short, and it was thought that one reason the treatment had only minimal success was that increased serum leptin levels were relatively short lived.

New trials were instigated using modified forms of leptin that would increase its half-life in the bloodstream. In the first of these studies to be published, the half-life of leptin was increased by pegylation (i.e. covalent linking of the leptin to polyethylene glycol polymers, which increases the half-life as well as decreases immunogenicity). Pegylated recombinant human leptin (PEG-OB) was given by SC injection on a weekly basis to a group of obese men at a dosage of 20 mg/week for 12 weeks.[62] Serum levels of PEG-OB averaged 200–300 µg/L, roughly ten times higher than endogenous leptin levels. However, the weight loss in the group treated with PEG-OB was similar to the weight loss in the placebo group. Despite the lack of effect on weight loss, the group treated with PEG-OB exhibited a reduction in appetite and hunger.[63] These results are consistent with leptin having several sites of action. PEG-OB acts at central sites controlling appetite but perhaps not at sites that control energy expenditure. There was no change in the RQ in the patients treated with PEG-OB, suggesting that this form of leptin had no effect on lipid oxidation. There were no differences in serum glucose and insulin levels or in insulin sensitivity. There was a larger reduction in serum triglycerides in the group treated with PEG-OB, which is consistent with a direct effect of leptin on peripheral lipid metabolism.

In a follow-up study, the dosage of PEG-OB was increased to 60 mg/week for 8 weeks but, again, there was no additional weight loss after 8 weeks in the group treated with PEG-OB compared with the placebo group.[64] In a further study reported at the recent International Congress on Obesity (ICO) in Brazil, PEG-OB 80 mg/week for 6 weeks resulted in greater weight loss than was seen with placebo.[65] However, in this study, the weight loss maintenance during the following 8 weeks was better in the placebo group, suggesting that leptin treatment may be beneficial in weight reduction, but it does not help in altering eating patterns, necessary for the maintenance of weight loss.

In another study, a different long-acting leptin, A-200, was given to a total of 200 obese patients for 24 weeks using three different treatment regimens (20 mg/day, 80mg thrice weekly, or 240mg once weekly).[66] At the end of the 24 weeks, changes in bodyweight and fat mass were similar in all three treatment groups; more patients in the groups treated with A-200 achieved a 10% reduction in bodyweight than in the placebo-treated group. Interestingly, the proportion of weight loss as a result of fat loss was greater in the groups treated with A-200 (79%) than in the placebo group (71%). Again, there was variability in the ability of the patients to respond to leptin treatment.

4.4 Limitations of Leptin in the Treatment of Obesity

In the limited number of trials of leptin treatment that have been reported, there has been little to encourage further investigation of leptin treatment as a therapeutic agent in the treatment of obesity.

All studies have reported minimal adverse effects of leptin treatment; however, the variability of response suggests that further work needs to be done to identify those patients in whom recombinant leptin administration may be an effective treatment option.

In none of the studies reported has leptin been given in a manner that reproduces normal patterns of circulating leptin. Circulating leptin exhibits a circadian pattern, with leptin levels usually highest around midnight.[67] Leptin is also normally released in a pulsatile pattern that is affected by a number of factors including circulating cortisol levels and dietary factors.[63] Therefore, the development of delivery strategies that can more closely mimic endogenous patterns of leptin release may prove more beneficial than the regimens used in the trials reported so far. Alternatively, strategies that enhance leptin transport into the brain or that bypass the blood-brain barrier and deliver leptin directly into the CSF could be beneficial.

4.5 Leptin as Therapy for Other Disorders

Leptin may prove to have greater therapeutic value in the treatment of disorders other than obesity. Trials using leptin in the treatment of lipodystrophy, where patients have low endogenous leptin levels consistent with their lack of both SC and visceral fat stores, have been more successful.[69,70] Lipodystrophy is characterized by insulin resistance, hypertriglyceridemia, and hepatic steatosis. In the first report, metreleptin was administered subcutaneously to nine patients, twice a day, for 4 months at escalating doses in order to achieve low, intermediate, and high physiologic replacement levels of leptin.[69] This regimen resulted in a significant decrease in glycosylated hemoglobin values, triglyceride levels, and liver volume. Patients were able to reduce antihyperglycemic therapy. There was also a decrease in reported daily caloric intake. In a subset of these patients, insulin sensitivity was shown to be increased and there was a reduction in hepatic and muscle triglyceride content, lending support to the hypothesis that intracellular lipid availability may modulate insulin action.[70] It is interesting that in these hypoleptinemic patients, leptin had an effect on glycemia that was not seen in the earlier trials of leptin in obese patients.[58,63]

5. Leptin Alternatives

5.1 Leptin Agonists

Another possible approach to the treatment of obesity is to use leptin-related peptide agonists (i.e. to construct peptide fragments corresponding to discrete domains within the leptin molecule). Rodent studies using different fragments have identified those regions of the leptin molecule that are able to reproduce not only the effects of leptin on weight gain and food intake, but also the effects on glycemic control.[71] These effects were also seen in the *db/db* mouse, suggesting that activation of the leptin receptor was not necessary for these peptides to be effective.[72] The clinical usefulness of these peptides still remains to be tested but suggests that leptin analogs that utilize signaling pathways distinct from those of leptin may have substantial clinical benefit, given the possible multitude of causes of leptin resistance in humans.

5.2 Ciliary Neurotrophic Factor

Another molecule that has shown potential as an antiobesity agent is ciliary neurotrophic factor (CNTF). In clinical trials of CNTF for the treatment of neurological disorders, it was found to cause significant weight loss. In subsequent studies in leptin-resistant mice with diet-induced obesity, CNTF was shown to reduce both food intake and bodyweight as well as ameliorate the hyperinsulinemia and hyperlipidemia seen in this model. CNTF induced hypothalamic STAT3 activation, whereas leptin had no effect, suggesting that CNTF was acting via nonleptin pathways.[73] Most importantly, the weight loss occurred without triggering any hunger signals or associated stress responses related to food deprivation, suggesting that unlike forced dieting, CNTF treatment does not result in binge overeating and rebound weight gain. Certainly, in the first human studies using Axokine™[1], a modified form of CNTF, not only was weight loss achieved but this weight loss was maintained for a further 12 months.[74] Axokine™ was given at a dosage of 1 μg/kg/day subcutaneously for 12 weeks, causing an average weight loss of 8.8kg. After a further 48 weeks, the average weight loss remained at 9.2kg. Phase III clinical trials of Axokine™ are now underway and trials using a pegylated form of Axokine™ have also commenced.

5.3 Neuropeptides in the Leptin Signaling Cascade

The discovery of leptin has led to an increase in our knowledge of the interaction between the different hypothalamic neuropeptides regulating energy homeostasis. Therefore, agents that target neuropeptides in the leptin signaling cascade could also have potential therapeutic benefit. However, there is sufficient evidence to suggest that in rodents at least there is considerable redundancy in these neurochemical systems such that the targeting of a single pathway may not be sufficient to produce any substantial effect on energy intake or weight loss. This indicates that any therapeutic benefit from the development of agonists or antagonists to recep-

1 The use of tradenames is for identification purposes only and does not imply endorsement.

tors in these pathways may lie in a combination of analogs rather than the targeting of a specific pathway.

6. Conclusion

This review has presented a summary of studies of leptin treatment in both animals and humans. Leptin itself appears to have limited clinical benefit as a treatment option for obesity. However, identification of the mechanisms at the molecular level by which leptin functions has provided new insights into the pathophysiology of obesity and will allow the development of new strategies to combat the ever-increasing problem of obesity.

Acknowledgements

No sources of funding were used to assist in the preparation of this manuscript. The authors have no conflicts of interest that are directly relevant to the content of this manuscript.

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Correspondence and offprints: Dr *Kim S. Bell-Anderson*, Human Nutrition Unit, School of Molecular and Microbial Biosciences, University of Sydney, Sydney, NSW 2006, Australia.
E-mail: kim@usyd.edu.au

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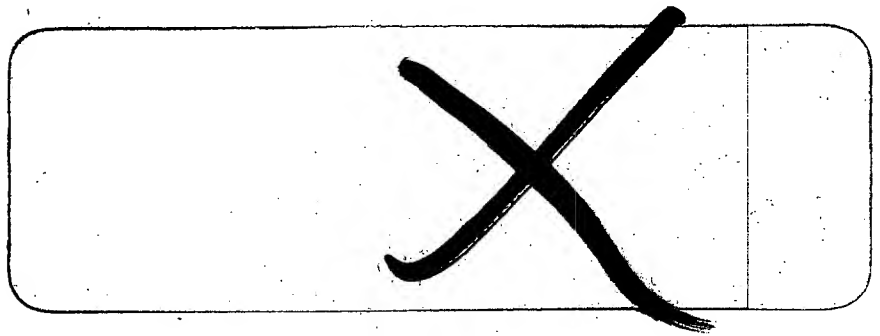

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